

Inhibiting Bacterial Fatty Acid Synthesis*

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The type II fatty acid synthase consists of a series of individual enzymes, each encoded by a separate gene, that catalyze discrete steps in chain elongation. The formation of fatty acids is vital to bacteria, and each of the essential enzymes and their acyl group carriers represent a potential target for the development of novel antibacterial therapeutics. High resolution x-ray and/or NMR structures of representative members of every enzyme in the type II pathway are now available, and these structures are a valuable resource to guide antibacterial drug discovery. The role of each enzyme in regulating pathway activity and the diversity in the components of the pathway in the major human pathogens are important considerations in deciding the most suitable targets for future drug development.

Fatty acid synthesis by the bacterial type II fatty acid synthase (FASII)² is carried out by a series of discrete enzymes diagrammed in Fig. 1 whose properties are extensively reviewed elsewhere (1, 2). Although the reactions catalyzed by the multifunctional type I synthase found in mammals are the same, there exist important structural differences in the enzymes that allow the identification of inhibitors that selectively target the bacterial system (for a summary, see Table 1) (3–5). The individual genes of bacterial fatty acid synthesis have all been cloned from the model organism *Escherichia coli* as well as from various other human pathogens. Generally, the enzymes are homologous, are readily identified using standard bioinformatics techniques, and share similar biochemical properties that improve the prospects for identifying broad spectrum antibacterials. Also, high resolution structures of all members of the pathway are now known (2), and this facilitates the structure-based design of new inhibitory compounds. Most of the FASII enzymes are essential for bacterial viability and are thus in principle suitable targets for antibacterial drug discovery. This review highlights the progress in the field and evaluates the suitability and future prospects for each pathway step as a drug discovery target.

Initiating Steps in FASII

The first committed step in fatty acid synthesis is the carboxylation of acetyl-CoA by acetyl-CoA carboxylase (ACC). This reaction is carried out by the interacting AccBC and AccAD complexes (6). Plants also have a type II fatty acid synthase, and plant ACC is the target for widely used commercial herbicides, which validates this step as an antibacterial target (7). The finding that broad spectrum pyrrolidinedione natural products, such as moiramide B, specifically target the AccAD complex (8) strengthens the argument for pursuing ACC inhibitors. The crystal structure of the yeast ACC carboxyltransferase domain and its complex with herbicides (9, 10) points the way to the rational design of inhibitors. Recently, *in silico* screening using the AccD5 structure of *Mycobacterium tuberculosis* identified one inhibitor with a K_i of 13 μ M (11). Currently, bacterial ACC remains one of the most promising and underexplored targets in type II fatty acid synthesis.

FabD, malonyl-CoA:ACP transacylase, catalyzes the rapid equilibrium of

malonyl groups between CoA and ACP. This enzyme is present in excess and does not have a defined regulatory role in the pathway. Although it is an essential gene, it is likely that an inhibitor would have to be almost 100% effective to be therapeutically useful. These considerations have dampened the enthusiasm for screening libraries for lead compounds against this enzyme. Although a natural product FabD inhibitor was recently identified (12), it remains unknown whether its antibacterial activity is actually related to its ability to block FabD activity.

FabH is essential for the initiation of fatty acid synthesis (13) and is a regulated step thought to play a key role in determining the amount of fatty acids produced by the pathway (14, 15). These properties have led FabH to be targeted for drug development by several groups. The most extensively evaluated case is the development of compounds that act against *Sp*FabH (16). Co-crystal structures of inhibitors with FabH aided in improving the design (17, 18). Although these inhibitors were potent against *Sp*FabH, they were not nearly as effective against *Ec*FabH or *Hi*FabH (16). He *et al.* (18) also note the wide differences in effectiveness of FabH inhibitors against different species of bacteria. These data probably reflect an important drawback to developing FabH as a target, namely that the enzyme has species-specific substrate specificities depending on whether the organism makes straight, branched, or very long chain fatty acids. In *E. coli*, the enzyme is selective for acetyl-CoA; in the Gram-positive pathogens like *Staphylococcus aureus* the FabH is primed by 4- or 5-carbon branched chain acyl-CoAs; and in *M. tuberculosis*, the enzyme uses medium to long chain acyl-CoA primers. Thus, the active site architectures are variable, particularly in the molecular volume and hydrophobicity of the acyl chain binding pocket (2).

Elongation Condensing Enzymes

The elongation condensing enzymes play a key role in the regulation of fatty acid biosynthesis, and the fact that natural products are known to target this step in the pathway (3, 19–21) clearly marks them as desirable targets for therapeutics development. Cerulenin was the first FabB/FabF inhibitor identified. The condensing enzyme catalyzes the opening of the epoxide ring of cerulenin and the covalent modification of the active site cysteine (22). Cerulenin has a 12-carbon acyl chain that associates with the hydrophobic channel that accommodates the hydrocarbon chain of the acyl enzyme intermediate (Fig. 2) (23, 24). The binding sites for the most recently described natural product condensing enzyme inhibitors are not known (20, 25, 26), but considering their hydrophobic structures, they most likely bind to the acyl chain pocket of the protein. The natural product receiving the most interest is thiolactomycin (TLM), a member of a group of thiolactone fatty acid synthesis inhibitors produced by a number of organisms (27, 28). The TLM binding mode to the condensing enzyme active site is completely different from cerulenin (24). Rather than associating with the acyl chain channel, TLM binds to the other side of the condensing enzyme active site in a position occupied by malonyl-ACP (Fig. 2). The isolation of TLM-resistant mutants that map to the malonyl side of the active site supports the structural work (29). Although TLM itself has only modest antibacterial activity (30), the intense interest in TLM derives from its favorable physical properties, its effectiveness in mouse infection models (31), and its broad spectrum activity against important pathogens such as *M. tuberculosis* (32) and malaria (33). TLM analogs with increased potency and more desirable pharmacokinetic properties are being sought by a number of groups, albeit with limited success (34–39). One of the problems in making progress in this area is the difficult chemical synthetic routes to TLM analogs (39, 40). It is clear that the discovery of new chemical scaffolds is needed to spur development of the condensing enzyme inhibitors. The established broad spectrum occurrence and rate-controlled function of the elongation condensing enzyme inhibitors mean that efforts in this area will not diminish in the future.

The Reductases

FabI, the enoyl-ACP reductase, is one of the most intensively explored pathway targets. The explosion of research in this area was triggered by the discoveries that isoniazid (41), a front-line treatment for tuberculosis, and triclosan (42, 43), a widely used commercial antibacterial agent, both target fatty acid synthesis at the FabI step. The mechanisms for FabI inhibition by isoniazid and triclosan are known in detail (42, 44), and these slow binding inhibitors form a high affinity, slowly dissociable ternary complex with NAD⁺ and FabI (45–50). This type of

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² The abbreviations used are: FASII, type II fatty acid synthase; ACP, acyl carrier protein; ACC, acetyl-CoA carboxylase; TLM, thiolactomycin; proteins in type II fatty acid biosynthesis (Fab) are designated as FabA, FabD, etc., and the genes are abbreviated as *fabA*; species-specific isoforms are designated using the genus-species abbreviation, i.e. *Ec*FabA, for *E. coli* FabA; CoA biosynthetic pathway components are designated, CoaA, CoaB, etc.

These clear successes in targeting the FabI component and the determinant role of FabI in completing each cycle of elongation (52) make this enzyme one of the most attractive targets for drug discovery, and the future of FabI inhibitors as therapeutics appears bright. However, the discovery of FabK (53), the enoyl-ACP reductase of *Streptococcus pneumoniae*, represents an important caveat. This triclosan-resistant enoyl-ACP reductase requires both NADH and FMN (54) and has no sequence similarity to the prototypical FabI. Thus, organisms that contain a FabK are resistant to FabI-specific drugs. Because *S. pneumoniae* is highly desirable to be included in a target profile, developers focused on discovering only broad spectrum antibacterials have ceased exploration of FabI as a viable target. However, FabI inhibitors are extremely potent against many important pathogens, such as multidrug-resistant *S. aureus* (55–57), a significant problem in hospitals that has spread into the community as evidenced from the recent outbreaks among professional football players (58). Clinically useful drugs against *S. aureus* are likely to arise from this line of development in the near future (59, 60).

FabG, the β -ketoacyl-ACP reductase, is highly conserved and widely expressed, and only a single isoform is known in bacteria. These features suggest that drugs targeting this enzyme would have broad spectrum antibacterial activity, but there is only a single report of polyphenol inhibition of FabG (61). However, plant polyphenols have multiple cellular targets, and thus these compounds are not specific inhibitors of FabG. Although FabG activity does not have a rate-controlling role in the pathway, the enzyme displays a functional conformational flexibility (62) that represents both an impediment and an opportunity for drug discovery. On one hand, it is difficult to model drugs into a flexible substrate binding pocket, but on the other hand, it offers the prospect of discovering slow binding inhibitors, such as triclosan for FabI (see above), that would be extremely potent.

Dehydratases and Isomerases

FabA, β -hydroxyldecanoyl-ACP dehydratase/isomerase, is an essential enzyme for the production of unsaturated fatty acids by many bacteria (63). Although mechanism-based FabA inhibitors were the first pathway inhibitors described (64), FabA has received little attention because of the limited distribution of *fabA* genes in bacteria. Several important human pathogens, like *S. aureus* and *S. pneumoniae*, do not possess a FabA homolog.

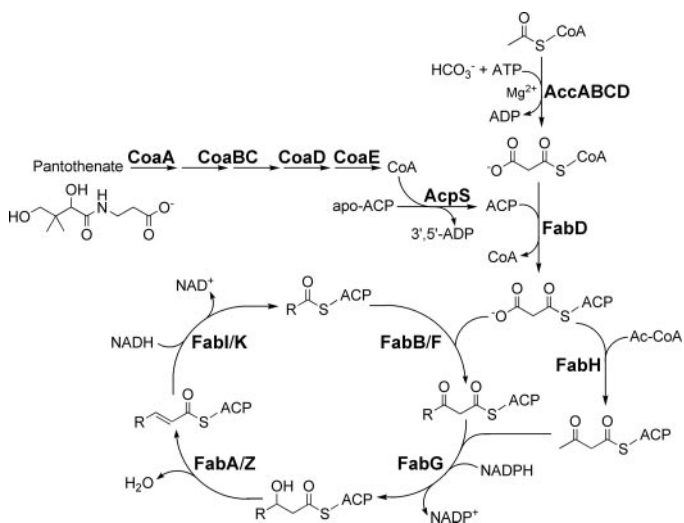


FIGURE 1. **The bacterial type II fatty acid biosynthetic pathway.** The production of membrane fatty acids in bacteria is catalyzed by a series of proteins encoded by separate genes that carry out the individual enzymatic steps depicted in the figure. See Table 1 for the activities of the FASII enzymes and Refs. 1 and 2 for recent reviews of the pathway.

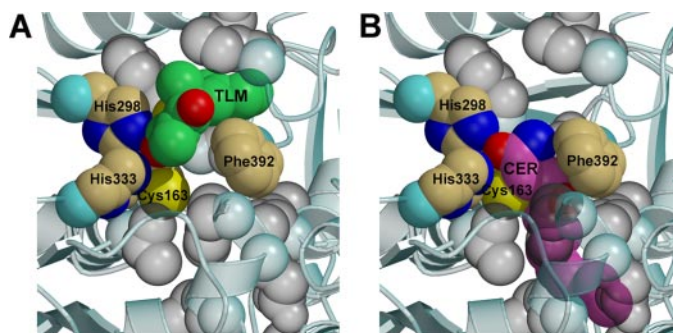


FIGURE 2. The FabbB-TLM and FabbB-cerulenin binary complexes. TLM and cerulenin bind tightly to opposite sides of the elongation condensing enzyme active site illustrated here by the FabbB-TLM and FabbB-cerulenin binary complexes shown in the same orientation (31). The side chains of the four key active site residues, Cys-163, His-298, His-303, and Phe-392, are shown in *tan* carbons, and other residues surrounding the active site are shown in semi-transparent *gray*. The secondary structure and the α carbons are shown in semi-transparent *blue*. *A*, TLM (shown in *green*) binds non-covalently and forms hydrogen bonds with the two histidines, but it does not enter the hydrophobic substrate-binding pocket. *B*, cerulenin (*CER*, shown in *magenta*) forms a covalent bond with the sulfhydryl of Cys-163 (shown in *yellow*) and two hydrogen bonds with the active site histidines, and the acyl chain extends down to occupy the hydrophobic substrate-binding pocket. The completion of the high resolution x-ray structures of all the enzymes of type II fatty acid synthesis (for review, see Ref. 2) makes a significant contribution to the mechanistic understanding and design of type II pathway inhibitors.

TABLE 1
Inhibitors of type II fatty acid biosynthetic enzymes

| Protein | Enzyme activity | Inhibitor | Ref. |
|---------|--|---------------------------|--------|
| AcpS | ACP synthase | Sch 538415 | 67 |
| | | 4 <i>H</i> -Oxazol-5-one | 70 |
| | | Anthranilic acid | 71 |
| AccABCD | ACC | Moiramide B | 8 |
| | | NCI 65828 | 11 |
| FabD | Malonyl-CoA:ACP transacylase | Corytuberine | 12 |
| FabH | β -Ketoacyl-ACP synthase III | HR12 (RW)-3981 | 98 |
| | | Indole analogs (SB418001) | 16, 17 |
| | | 1,2-Dithiole-3-ones | 18 |
| | | Benzoylaminobenzoic acids | 99 |
| FabB/F | β -Ketoacyl-ACP synthase I/II | Cerulenin | 22 |
| | | Thiolactomycin | 27, 28 |
| | | BABX | 20, 21 |
| | | Phomallenic acids | 25, 26 |
| FabG | β -Ketoacyl-ACP reductase | Polyphenols | 61 |
| FabA | β -Hydroxydecanoyl-ACP dehydratase | Allenic acids | 64 |
| | | 3-Decynoyl-NAC | 100 |
| FabZ | β -Hydroxyacyl-ACP dehydratase | NAS-91, NAS-21 | 65 |
| FabI | Enoyl-ACP reductase I | Isoniazid | 41 |
| | | Triclosan | 42, 43 |
| | | Diazaborine | 51 |
| | | Aminopyridines | 55, 57 |
| FabK | Enoyl-ACP reductase II | Aminopyridine | 55 |
| CoaA | Pantothenate kinase | Pantothenamides | 73–75 |
| CoaD | Phosphopantetheine adenylyltransferase | Dipeptide | 79 |

It seems that the most attractive candidate for drug development in this group of enzymes is FabZ. This protein is uniformly expressed in type II systems, and thus potent inhibitors would be broad spectrum antibiotics. Two inhibitors have been reported to target the FabZ of *Plasmodium falciparum* (65), but the efficacy of these compounds is unknown. A drawback to targeting FabZ is that it catalyzes a reversible reaction with the equilibrium lying on the side of the β -hydroxyacyl-ACP, and the activity of FabI is therefore required to pull cycles of elongation to completion (14, 52). The fact that the FabZ dehydratase does not catalyze a rate-determining step in the pathway diminishes its desirability as a candidate for drug discovery.

Targeting ACP and CoA

ACP is the acyl group carrier in type II fatty acid synthesis and has attracted attention as a target for drug development. A key feature of this protein is that the gene product is an apoprotein that is converted to the active form by the attachment of a 4'-phosphopantetheine prosthetic group from CoA by AcpS. This route to biosynthesis has been exploited for the development of inhibitors by targeting either AcpS or employing antimetabolites to inactivate ACP via their incorporation into CoA. A third approach is to simply block CoA biosynthesis, which would not only blunt fatty acid formation but also other metabolic processes that require CoA.

AcpS is widely expressed, and although some organisms have ancillary proteins that modify the ACPs of polyketide synthesis that can also use apo-ACP of FASII as substrate (66), AcpS is in many cases an essential gene in bacteria. One of the encouraging findings is the isolation of a natural product that inhibits AcpS with antibacterial activity against *S. aureus* (67). The high resolution structures of several AcpS proteins are known (68, 69), and structure-based design has been employed to identify a class of anthranilic acid inhibitors (70, 71). AcpS appears an excellent target that deserves continued attention.

The pantothenamide class of antibacterial agents were discovered in the 1970s (72) but only recently has their mechanism of action been elucidated. The first step was the discovery that pantothenate antimetabolites were not inhibitors of pantothenate kinase (CoaA) but were substrates for the enzyme that were subsequently incorporated into inactive CoA analogs (73). The next key finding was that these compounds actually exert their antibacterial effects through the incorporation of CoA analogs into ACP leading to its inactivation (74). The pantothenamides are particularly effective against *S. aureus*, and this is most likely because of their incorporation into multiple acyl carrier proteins (75). This antibacterial strategy relies on the phosphorylation of the antimetabolite by pantothenate kinase, followed by its incorporation by the enzymes of CoA biosynthesis into CoA analogs. The utility of these compounds is complicated by an unknown structural requirement for transport into the bacteria. Although a series of analogs are known that fulfill all of the requirements for utilization by the pathway enzymes, they are not effective as antibacterial agents because of their inability to penetrate the cell (76). Also, there are three distinct forms of CoaA expressed in bacteria. Types I and II utilize the pantothenamides as substrates (73, 75), but the type III CoaA does not (77), making bacteria species that solely express this isoform intrinsically resistant to pantothenate antimetabolites. Nonetheless, the pantothenamides characterized to date are very effective against drug-resistant *S. aureus* and have the potential to be developed further.

CoaD, the 4'-phosphopantetheine adenyltransferase, is the most logical target for developing antibacterials that target CoA biosynthesis. Detailed bioinformatic analysis of the CoA pathway shows that only a single CoaD isozyme is present in bacteria, and its sequence predicts that it is distinctly different from the multifunctional mammalian protein that carries out the same reaction (78). There is only a single publication reporting CoaD inhibitors (79), but the attractiveness of the target and the availability of high quality x-ray structures (80–82) suggest that CoaD inhibitors should receive greater attention.

Perspectives

The rising tide of multidrug-resistant pathogens ensures that the search for new antibiotics will continue. Type II fatty acid synthesis will remain an important component of this work because the pathway is essential, and cross-resistance to existing drugs that target other pathways is minimal. Fatty acid synthase drugs are likely to have their most immediate impact on combating methicillin-resistant *S. aureus* infections because of the sensitivity of this organism to both FabI inhibitors (60) and pantothenamide antimetabolites (75). The discovery of a type II fatty acid synthase system in other important human pathogens, such as the malarial parasite (*P. falciparum*), has spurred additional research into the identification of pathway inhibitors (83–85). Also, fatty acid synthesis inhibitors hold promise for the treatment of cancer (86–88), because inhibitors of fatty acid synthase trigger apoptosis in transformed cells, particularly those associated with breast and prostate

tumors. Human metabolic syndromes and obesity (89, 90) represent other important medical areas that will be impacted by the more efficacious type I fatty acid synthase inhibitors because of the recent discovery of regulation of food intake by malonyl-CoA (91–94).

The implementation of *in vivo* assays for fatty acid synthesis inhibitor screening will be important to future drug design efforts. Although *in vitro* screening of compound libraries against the individual enzymes invariably identifies chemical scaffolds that can be engineered to be more potent, the problems associated with cell wall/membrane permeability and efflux pumps are critical to overcome and are not addressed by this approach. Some initial work toward *in vivo* assays for FASII inhibitor screening has been recently reported (25, 95), and novel applications of this general approach promise to more accurately identify suitable natural product lead compounds, particularly against the elongation condensing enzymes (95). The use of microarray gene expression analysis to evaluate the mechanism of action is another important tool that will see increased use in future drug target analysis. Although a specific chemical scaffold may inhibit the target enzyme of interest *in vitro*, it is possible and maybe even probable that the antibacterial property will be due to the effects of the derivatives of the parent compound on another biochemical target *in vivo*. Even if a compound is validated as a fatty acid synthesis inhibitor by several criteria, the production of a combinatorial library of compounds may lead to more potent antibacterial agents and at the same time migrate away from the original mechanism of action. There are many reports in the field where this important consideration has not been addressed. Two recent reports on the categorization of antimicrobial targets based on genome-wide expression analysis hold promise that the pathways being affected by an antibacterial compound of an unknown mechanism of action can be identified from its effects on global transcriptional profiles through comparison to databases established using compounds of known mechanisms (96, 97).

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